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PRODUCTION AND ISOLATION OF POLYSACCHARIDES FROM MYCELIA OF NATURAL ISOLATES OF HIGHER FUNGI

PROIZVODNJA I IZOLACIJA POLISAHARIDA IZ MICELIJA PRIRODNIH IZOLATA VIŠIH GLJIVA

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ABSTRACT

The traditional use of higher fungi in medicine and nutrition is largely attributed to their rich content of bioactive polysaccharides. This study evaluated the ability of four fungal isolates: Ganoderma resinaceum NMKSS, Bjerkander aadusta TMF1, Fomes fomentarius TMF2 and Ganoderma sp. to produce intracellular (IPS) and extracellular polysaccharides (EPS) during submerged fermentation using glucose as the carbon source. Polysaccharides were extracted from dried mycelial biomass and culture supernatants, respectively, and quantified via the phenol-sulfuric acid assay. Initial screening revealed that EPS yields ranging from 0.11 to 0.76 mg/ml and IPS yields between 0.5 and 113.3 mg/g dry biomass. Notably, B. adusta TMF1 and F. fomentarius TMF2 exhibited significantly higher polysaccharide production and were selected for further work. The influence of organic nitrogen sources on polysaccharide yield was assessed, demonstrating that peptone supplementation maximized both EPS and IPS production. F. fomentarius TMF2 achieved the highest EPS yield of 0.84 mg/mL, conversely, B. adusta TMF1 produced a maximum IPS yield of 134.1 mg/g dry biomass. Yeast extract favored biomass accumulation but was less effective in stimulating polysaccharide synthesis. These findings suggest that nitrogen source composition critically influences the balance between fungal growth and polysaccharide production. Further research is needed to optimize culture conditions and fully exploit the biotechnological potential of these isolates for food and pharmaceutical applications.

Keywords: *higher fungi; intracellular polysaccharides; extracellular polysaccharides.*

REZIME

Tradicionalna upotreba viših gljiva u medicini i ishrani u velikoj meri se pripisuje njihovom bogatom sadržaju polisaharida. Ovo istraživanje bavi se ispitivanjem potencijala četiri nova izolata viših gljiva - Ganoderma resinaceum NMKSS, Bjerkandera adusta TMF1, Fomes fomentarius TMF2 i Ganoderma sp. - da proizvode intracelularne (IPS) i ekstracelularne polisaharide (EPS) tokom submerzne fermentacije u podlozi sa glukozom kao izvorom ugljenika. Polisaharidi su ekstrahovani iz osušene micelijske biomase (IPS), odnosno fermentisanog bujona (EPS) i kvantifikovani pomoću fenol-sumporne metode. Početni skrining je pokazao da se prinosi EPS kreću od 0,11 do 0,76 mg/ml, a prinosi IPS između 0,5 i 113,3 mg/g suve biomase. Izolati,B. adusta TMF1 i F. fomentarius TMF2 pokazali značajno veću proizvodnju polisaharide i odabrani su za dalji rad. U narednom koraku, procenjen je uticaj organskih izvora azota na prinos polisaharida. Suplementacija peptonom kao jedinim izvorom azota najviše je uticala na povećanje proizvodnje i EPS i IPS. F. fomentarius TMF2 je postigao najveći prinos EPS-a od 0,84 mg/ml. Nasuprot tome, B. adusta TMF1 dala je maksimalni prinos IPS-a od 134,1 mg/g suve biomase. Ekstrakt kvasca je favorizovao akumulaciju biomase, ali je bio manje efikasan u stimulisanju sinteze polisaharida. Ovi rezultati ukazuju na to da sastav izvora azotaima uticaja na ravnotežu između rasta biomase gljiva i proizvodnje polisaharida. Potrebna su dalja istraživanja kako bi se optimizovali uslovi gajenja i u potpunosti iskoristio biotehnološki potencijal ovih izolata za primenu u proizvodnji polisaharide i potencijalno u prehrambenoj i farmaceutskoj industriji.

Ključnereči*: više gljive, intracelularni polisaharidi, ekstracelularni polisaharidi.*

INTRODUCTION

Mushrooms have been integral to traditional remedial practices across diverse Asian cultures for centuries, where they have been esteemed not only as dietary components but also for their diverse therapeutic applications. Within systems such as Traditional Chinese Medicine, these fungi have historically been utilized to promote general health, enhance longevity, and treat a range of pathological conditions. In recent decades, there has been a marked increase in scientific interest within Western biomedical research regarding the pharmacological potential of medicinal mushrooms. A growing body of evidence has identified these fungi as a rich source of bioactive compounds, including but not limited to immunomodulators, prebiotics, antimicrobial agents, antioxidants, anticarcinogenic, and antidiabetic substances. These properties are largely attributed to their high content of structurally diverse polysaccharides, particularly β-glucan, which have been demonstrated to play a central role in modulating innate and adaptive immune responses (Zhong et al., 2023), as well as exerting other significant physiological effects. Although extensive research has been conducted on well-known species such as *Ganoderma lucidum* (Dong et al., 2012; Yang et al., 2025), *Lentinula edodes* (Vetvicka & Vetvickova, 2014), and *Grifola frondosa*, the broader fungal kingdom remains vastly underexplored.

Recent estimates suggest that there are between 2.2 and 3.8 million fungal species globally, of which fewer than 10% have been formally described (Hawksworth & Lücking, 2017). This immense biodiversity represents a largely untapped reservoir of novel bioactive molecules. Isolating and testing new fungal strains, particularly from unique or extreme environments, hold great promise for the discovery of polysaccharides with distinct structural and functional characteristics.

The biological activity of fungal polysaccharides is closely linked to their chemical structure, including molecular weight, branching patterns, glycosidic linkages, monosaccharide composition, and conformational features such as triple-helix formation. These structural attributes can significantly influence the polysaccharides' ability to modulate the immune system, combat oxidative stress, inhibit tumor growth, and regulate blood glucose levels. Given their diverse bioactivities and low toxicity, fungal polysaccharides have become strong candidates for the development of new therapeutic agents, functional foods, and nutraceuticals (Sun et al., 2022; Wang et al., 2022). However, the discovery of novel polysaccharides requires a systematic approach that includes the isolation of new fungal strains, extraction and purification of polysaccharides, structural characterization, and evaluation of biological activity through both in vitro and in vivo studies.

Traditionally, the primary reservoir of these bioactive polysaccharides has been the fruiting body of the mushroom. However, limitations related to cultivation time, environmental variability, and standardization has prompted the exploration of alternative production systems. Submerged cultivation of fungal mycelia has emerged as a sustainable and scalable biotechnological method for the controlled production of polysaccharides under standardized conditions (Berger et al., 2022). In such liquid culture systems, fungi synthesize both intracellular polysaccharides (IPS), retained within the mycelial biomass, and exopolysaccharides (EPS), which are secreted into the surrounding culture medium.

Table 1. Nutrient brought composition for submerged fermentation of fungi

Nutrient, g/l	Liquide medium 1	Liquide medium 2	Liquide medium 3
Glucose	50	50	50
Yeast extract	5	2.5	0
Peptone	0	2.5	5
KH_2PO_4	2.5	2.5	2.5
$MgSO_4*7H_2O$	1.25	1.25	1.25
KCl	0.5	0.5	0.5

In addition to polysaccharide production, fungal mycelium has garnered attention for its industrial potential in producing biodegradable mycelium-based materials offering environmentally friendly alternatives to synthetic materials, exhibiting advantageous mechanical properties such as biodegradability, fire resistance, and acoustic insulation (Krsmanović et al., 2024).

The exploration and testing of novel fungal isolates represent a critical step in the discovery and development of natural therapeutic agents, particularly in the context of polysaccharide extraction. Therefore, the current study was aimed to explore the polysaccharide-producing potential of newly isolated fungal strains. In this research we tested production potential of four new natural isolates of fungi, by growing their mycelia under submerged conditions.

MATERIAL AND METHOD

The mycelia of four higher fungi *Ganoderma resinaceum* NMKSS, *Bjerkardera adusta* TMF1, *Fomes fomentarius* TMF2 and *Ganoderma* sp., described in our previous work (Ilić et al., 2023) were assessed for polysaccharide production. In order to investigate their ability to produce EPS and IPS, they were grown in modified El-Naghy medium (glucose 50.0 g/L; $NaNO_3$ 2.5 g/L; $(NH_4)_2HPO_4$ 2.5 g/L; KH_2PO_4 2.5 g/L; $MgSO_4$ 1.25 g/L; KCl 0.5g/L and pH 5.3) (Prajapati et al., 2022), on an orbital shaker (120 rpm) at 30±2 °C for 10 days.

Isolation and purification of intracellular (IPS) and extracellular (EPS) polysaccharides

The biomass was separated by centrifugation (6.000 rpm/min) and washed with distilled water. The biomass was distributed in Petri dishes in a thin layer and left in a dryer for two days, at 45 °C (with the airflow set to 100%), until it was completely dry. Afterwards, all samples were ground using a ball mill (50s at a frequency of 30Hz) and their mass was measured on an analytical balance for biomass assessment. These samples were further processed for the isolation of IPS. Briefly, the dried mycelium was extracted with hot water, (at 90°C for 2 h), and the resulting aqueous extract was subjected to cold precipitation by the addition of four volumes of ethanol. The precipitation step was performed at low temperature to ensure efficient recovery of IPS, which was subsequently collected and used for further analyses. The EPS were obtained by cold ethanol precipitation directly from the supernatants.

After the first set of experiments, it was determined which of these four fungi has the highest content of IPS and EPS. Based on these results, *B. adusta* TMF1 and *F. fomentarius* TMF2 strains were selected for further work. In the next phase of research, the selected strains were grown under the same conditions (30±2 °C, 120 rpm for 10 days) with varying sources of organic nitrogen (yeast extract, pepton and yeast extract and peptone). Compositions of medium broth are listed in Table 1.

Content of produced EPS and IPS was estimated colorimetrically by phenol–sulfuric acid method using glucose as a standard (Dubois et al., 1956). The absorbance was measured at 490 nm in spectrophotometer (Ultrospec 3300 proAmersham Bioscience). The results are expressed as g glucose equivalents per L for EPS, and as mg glucose equivalents per g of biomass dry weight for IPS.

Statistical analysis

The results are presented as means ± standard deviations. Statistical analysis was conducted using OriginPro 9.0 (OriginLab Corporation). Differences between experimental groups were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's test. A confidence level of 95% was considered statistically significant.

RESULTS AND DISCUSSION

Four isolates were primarily screened for production of IPS, EPS and biomass in modified El-Naghy culture media. The first screening revealed the polysaccharide production potential in three of four tested strains (Fig 1). IPS yield was in range 0.5 – 113.3 mg/g. The concentration of IPS from *Ganoderma sp.* was significantly lower (0.5mg/g) compared to the others, while *B.adusta* TMF1 resulted in the highest concentration of 113.3 mg/g for IPS. Similar results were obtained for EPS. The EPS yield was in the range 0.11- 0. 76 g/L, while the concentration of EPS from the *Ganoderma sp.* was the lowest (Fig 1). *B. adusta* TMF1 and *F. fomentarius* TMF2 strains had higher yield of EPS compared to other strains, 0.53 g/L and 0.76 g/L respectively (Fig 1.).

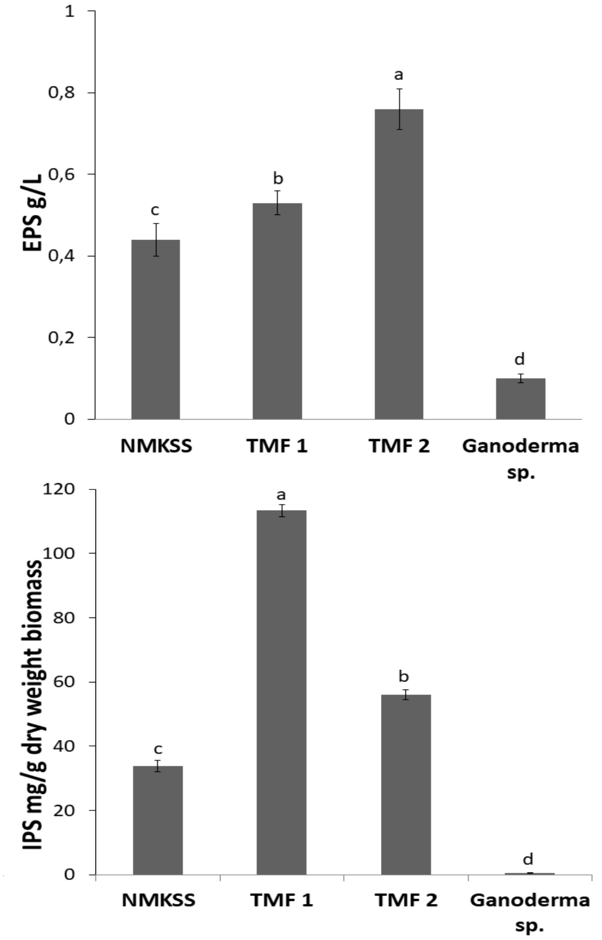

Fig. 1.a EPS and Fig 1b. IPS yield results after first screening among four natural isolates of fungi: Ganoderma resinaceum NMKSS, Bjerkardera adusta TMF1, Fomes fomentarius TMF2, and Ganoderma sp. Significant differences between the samples are labeled with different letters.

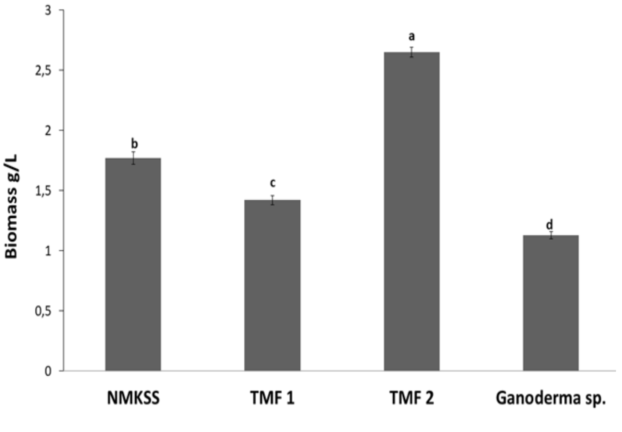

Fig. 2. Biomass concentrations after first screening for polysaccharides producers among four natural isolates of fungi: Ganoderma resinaceum NMKSS, Bjerkardera adusta TMF1, Fomes fomentarius TMF2, and Ganoderma sp.. Significant differences between the samples are labeled with different letters.

Based on the results from Fig. 2 modified El-Naghi medium as a growth medium was the most suitable for *F. fomentarius* TMF2. which achieved the highest biomass yield (2.67 g/L). This result is significantly higher compared to other fugal isolates, 1.77 g/L for *G. resinaceum* NMKSS, 1.42 g/L for *B. adusta* TMF1 and 1.13 g/L for *Ganoderma* sp. Overall, taking into account the results for IPS and EPS and biomass, it can be concluded that under the given experimental conditions, *B. adusta* TMF1 and *F. fomentarius* TMF2 have the highest potential for polysaccharide production. Further experimental work was continued with these two strains.

It is well known that fungal growth is largely influenced by the type and availability of nitrogen in the substrate medium. Inorganic sources (like ammonium) often supporting faster initial growth, while organic sources (hitin, peptone, amino acids) rather support robust grow. In order to investigate the effect of organic nitrogen source on IPS and EPS production, mycelia of two fungi *B. adusta* TMF1 and *F. fomentarius* TMF2 were grown in three liquid media which differed only in nitrogen source, peptone and yeast extract. These compounds are widely used in microbiology and biotechnology as essential and complex nutrients in cultivation/culture media because they are rich in peptides, vitamins etc.

Regarding the EPS yield, medium with peptone as a nitrogen source (Medium 3) was the best choice for both fungi (Fig 3a. and 3b.). The *F. fomentarius* TMF2 (0.84 g/L) gave significantly better results compared to *B. adusta* TMF1 (0.54 g/L). The enhancement of EPS produced by *F. fomentarius* TMF2 was 45% and 22% comparing with medium containing yeast extract only (Medium 1) (0.46 g/L) and yeast extract and peptone (Medium 2) (0.66 g/L), respectively. This result for *F. fomentarius* TMF2 was 11% better compared to result obtained after first screening (Fig. 1a) in which inorganic nitrogen served as the sole nitrogen source, while in the case of the *B. adusta* TMF1 strain, the increase in EPS concentration was negligible.

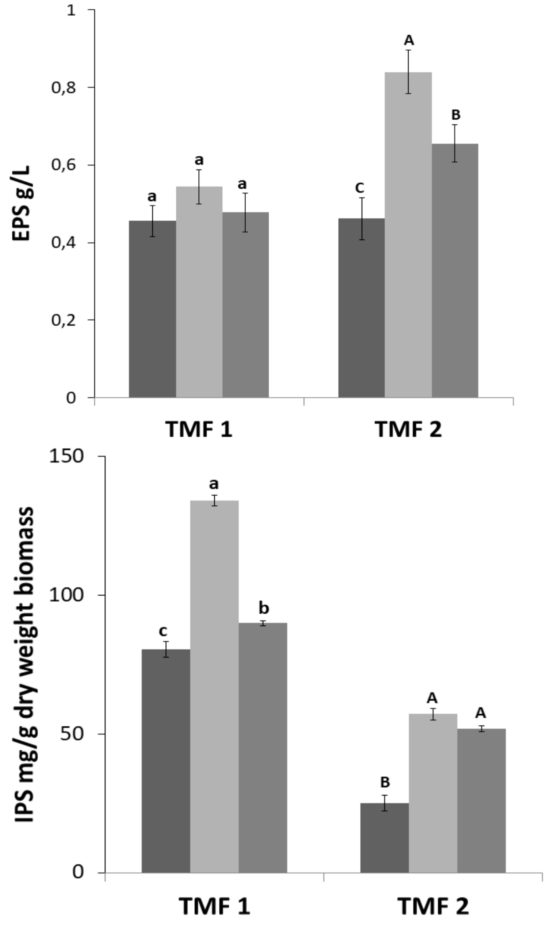

Fig. 3a. EPS and Fig 3b. IPS yield results for Bjerkardera adusta TMF1 and *Fomes fomentarius* TMF2 *in three different broth media which differed only in organic nitrogen source: yeast extract (Medium 1) (dark gray), peptone and yeast extract (Medium 2) (light gray) and peptone (Medium 3) (medium gray). Significant differences between the samples are labeled with different letters.*

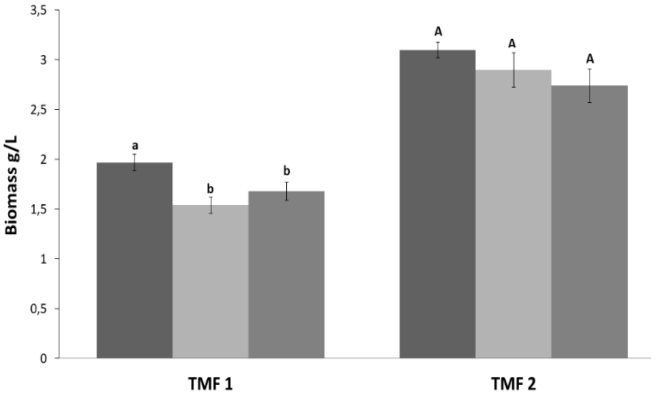

Fig. 4. Biomass yield forBjerkardera adusta TMF1 *and Fomes fomentarius* TMF2 *in three different broth media which differed only in inorganic nitrogen source: yeast extract (dark gray), peptone and yeast extract (light gray) and peptone (medium gray). Significant differences between the samples are labeled with different letters.*

As far as the IPS yield results, it was reversed, and the best outcome was for *B. adusta* TMF1 (134.2 mg/g) (Fig 3b.). This result was almost 20% better compared to one obtained after the first screening (Fig 1b). The best result for IPS for *F. fomentarius* TMF2 was twice time lower compared with *B. adusta* TMF1 yield, 57.1 mg/g. For this strain, there was almost no difference in yield after first and second screening.

Most studies on fungal polysaccharide production and optimization of cultivation conditions evaluate only EPS as the principal output parameter. However, in the present study, we aimed to identify the optimal culture factor, specifically the nitrogen source, for the simultaneous production of both polysaccharide types, EPS and IPS. Based on these results, it can be concluded that peptone was the most suitable nitrogen source for polysaccharide production in both *B. adusta* TMF1 and *F. fomentarius* TMF2 strains. Cultivation of the *B. adusta* TMF1 strain in a medium containing peptone as the sole nitrogen source (Medium 3) resulted in a significant increase in IPS production, whereas the yield of produced EPS was comparable to that obtained in a medium supplemented with inorganic nitrogen. Overall, considering the total polysaccharide yield, peptone expressed the most prominent effect on polysaccharide production. According to obtained results, a similar conclusion can be drawn for strain *F. fomentarius* TMF2, indicating that peptone is also the most suitable nitrogen source for this strain. In the case of the *F. fomentarius* TMF2, supplementation of the medium with peptone resulted in a significant increase in EPS yield, whereas no differences were observed in IPS production. Similarly, to the *B. adusta* TMF1, the total polysaccharide yield was significantly higher in the medium containing peptone (Medium 3) compared to the medium supplemented with inorganic nitrogen.

The increase in polysaccharide production was followed by the trend of increasing biomass. For both tested strains, *B. adusta* TMF1 and *F. fomentarius* TMF2, biomass increased in range of 5 to 15% compared to the results in first screening (Fig 2). For *B. adusta* TMF1 biomass yield was in range of 1.54 to 1.97 g/L and for *F. fomentarius* TMF2 it was from 2.74 to 3.1 g/L (Fig. 4). Contrary to polysaccharide production, the highest increase in biomass for *B. adusta* TMF1 and *F. fomentarius* TMF2 was in medium with yeast extract as a sole nitrogen source (Medium 1), 1.97 g/L and 3.1 g/L, respectively. These findings suggest that both mycelial growth and polysaccharide production are strongly influenced by the type of nitrogen source. Yeast extract and peptone, as organic nitrogen sources, are not only more complex than inorganic sources but are also rich in additional nutrients. Yeast extract is abundant in vitamins, minerals, and complex proteins, which act as growth-promoting factors, whereas peptone primarily consists of protein hydrolysates that are more readily utilized by microorganisms, thereby directly supporting polysaccharide synthesis.

Similar findings regarding the influence of different nitrogen sources on biomass and polysaccharide production have been reported by other authors. Chang et al. (Chang et al., 2006) investigated the effect of various nitrogen sources on biomass and EPS production during submerged cultivation of *Ganoderma lucidum*. Among the five nitrogen sources tested, yeast extract resulted in the highest biomass yield (2.2 g/L), which is consistent with our findings. Furthermore, by optimizing additional cultivation parameters, such as the carbon source, and supplementing the medium with additional components, the authors achieved up to an eightfold increase in yield within the same study.

On the other hand, organic nitrogen sources are typically associated with high costs, prompting the growing use of more cost-effective alternatives such as agro-industrial residues. While these ”waste” substrates are predominantly employed as carbon sources (Pilafidis et al., 2024), there is evidence in the literature of their supplementation in culture media as alternative nitrogen sources. Ogidi and co-workers (Ogidi et al., 2020), found that agro-industrial residues (fruit peels, coconut husk, groundnut shell and walnut shell) used as supplements in submerged culture had a significant positive effect on exopolysaccharide (EPS) production by *Pleurotus pulmonarius.*

Results from this study suggest a good potential for both strains, *B. adusta* TMF1 and *F. fomentarius* TMF2, to produce polysaccharides. In order to investigate their full potential further experiments should be conducted, towards the optimization of some other components of the nutrient medium or supplementation with some additives. Beside nitrogen source, reducing sugars can also be investigated as a part of optimization process. For example, in the case of *Ganoderma tsugae*, after optimization on lactose-based medium (instead of glucose) at 28°C, the peak of EPS yield was 1.21g/L (Serody et al., 2024). This was for almost 35% higher than in medium with glucose, so the authors have concluded that this strain utilizes lactose rather than glucose. Furthermore, medium additives have been shown to affect the synthesis of active products in fungi, among them and polysaccharides as well. The Li and co-workers investigated the effects of corn stalk, poplar sawdust, Tween-80, and oleic acid on mycelia biomass and physicochemical properties, as well as the bioactivity of polysaccharides, in the submerged culture of *Bjerkandera fumosa* (Li et al., 2024). Maximum polysaccharides yield was obtained in medium supplemented with oleic acid and corn stalk. However, there results have shown that supplementation of medium with different additives affects the length of the polysaccharide chain as well as the monosaccharide composition.

CONCLUSION

The present study demonstrated that among four newly isolated fungal strains, *Bjerkandera adusta* TMF1 and *Fomes fomentarius* TMF2 exhibited the highest potential for polysaccharide production under submerged cultivation conditions.

Peptone was identified as the most suitable nitrogen source for enhancing total polysaccharide yield. *B. adusta* TMF1 showed a pronounced increase in IPS production (134.2 mg/g), whereas *F. fomentarius* TMF2 exhibited enhanced EPS synthesis (0.84 g/L).

In contrast, yeast extract promoted biomass accumulation but did not maximize polysaccharide production.

These findings highlight the importance of nitrogen source selection in regulating the balance between fungal growth and polysaccharide biosynthesis. Further optimization of cultivation parameters is required to enhance yield and evaluate the structural and biological properties of the produced polysaccharides.

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